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Germ Cells and Gametogenesis

Program/Abstract # 338

Mechanism of global gene silencing and reactivation during oocyte growth at the one-cell stage after fertilization in mice

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In mice, transcription ceases globally with the condensation of chromatin during oocyte growth and it remains silent for a while after fertilization. Transcription is reactivated at the mid one-cell stage. We investigated the involvement of the largest RNA polymerase II subunit (RNAP II) in the mechanism regulating this global transcriptional silencing and reactivation. Immunoblotting and immunocytochemistry using antibodies against phosphorylated and unphosphorylated forms of RNAP II revealed that in the small growing oocytes and late one-cell stage embryos in which active transcription occurs, RNAP II was associated with DNA and was phosphorylated at serine 2 and serine 5, which is generally observed in somatic cells. By contrast, in transcriptionally inactive full-grown oocytes and early one-cell embryos, RNAP II was dissociated from DNA and phosphorylated in a pattern differing from that in the transcriptionally active cells. These results suggest that transcriptional silencing is caused by the dissociation of RNAP II from DNA and its different phosphorylation pattern from somatic cells halts the association with DNA. In addition, RNAP II was even dissociated from DNA in trichostatin A-treated full-grown oocytes in which the chromatin was decondensed, suggesting that chromatin condensation is not an essential process in gene silencing during oocyte growth.

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Program/Abstract # 339

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Program/Abstract # 340

Roles of natriuretic peptide receptor 2 and a novel gene in skeletal development, ovulation, and spermatogenesis

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Skeletal growth is an important component of postnatal development, as it determines an individual's final stature. Disruptions in this process result in skeletal dysplasia. We mapped and characterized two recessive, spontaneous mouse mutants with skeletal dysplasia and fertility problems, *peewee* (*pwe*) and *chagun* (*cha*). *Pwe* is caused by a frameshift and premature stop codon in *Npr2* causing loss of function of natriuretic peptide receptor 2. *Npr2* is a guanylyl cyclase activated by C-type natriuretic peptide (CNP) to stimulate endochondral ossification. The *pwe* growth defect is evident embryonically and can be corrected in cultures of fetal tibiae by administration of the MEK1/2 inhibitor U0126. *Pwe* females are infertile and exhibit ovarian hypoplasia. *Npr2* is expressed in many tissues of the HPG axis and has been proposed to regulate follicular atresia in the ovary. Studies are underway to define the role of *Npr2* in pituitary gonadotropin production and ovulation. The *chagun* critical interval contains 3 known genes that we are screening for mutations. *Cha* males are infertile due to progressive loss of germ cells and present vacuolated seminiferous tubules similar to Sertoli cell-only syndrome. *Cha* is embryonic lethal on the BL6 background. Identification of the causative mutation and its modifier(s) in mice will provide candidate genes for skeletal growth and male fertility defects of unknown etiology in humans and add to our basic understanding of bone and gonad development.

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Program/Abstract # 341

Src family kinase signaling during egg maturation and fertilization in a marine protostome worm

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Whether Src family kinases (SFKs) are required for the onset of egg maturation (=germinal vesicle breakdown, GVBD) or fertilization-induced Ca2+ signals in protostomes such as arthropods, molluscs, and worms has not been widely analyzed. Thus, oocytes of the marine nemertean worm *Cerebratulus* were pre-incubated in calcium-free seawater (CaFSW) to block maturation before being treated with PP2 or U73122 (U7) to block SFKs or downstream phospholipase C activity, respectively. After immersion in seawater (SW) to trigger maturation, oocytes incubated with PP2 or U7 continued to undergo GVBD as in SW-stimulated controls, and in fact such drug treatments by themselves slightly elevated maturation levels over those exhibited in CaFSW alone. Accordingly, SFK activity did not markedly rise during maturation based on blots using a phospho-specific SFK antibody. In subsequent fertilizations, mature metaphase-I-arrested eggs that had been treated with PP2 or U7 displayed lower post-insemination levels of polar body formation and cleavage than in controls. However, such

reductions by PP2 and U7 may have been generated via different mechanisms, given that the two drugs had varying effects on sperm incorporations and pronuclear differentiations. Moreover, confocal imaging revealed Ca2+ oscillations were blocked by U7 but not by PP2. Collectively, such data fail to support the view that SFK signaling is required for either GVBD or for initiating fertilization-induced Ca2+ oscillations in *Cerebratulus* and instead suggest that PP2-mediated inhibitions of polar body formation and cleavage involve undetermined drug effects on processes other than oscillation generation.

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Program/Abstract # 342

The mammalian Doublesex homolog DMRT1 controls the mitosis versus meiosis decision in males

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Germ cells are uniquely capable of undergoing either mitotic divisions, like other cells, or meiotic divisions that permit gametogenesis. In mammals meiosis is triggered by retinoic acid (RA), which activates genes including the meiotic inducer *Stra8*. Fetal males avoid meiosis by degrading RA in the fetal testis. When meiosis begins in males at puberty it requires RA and *Stra8*, but how these are controlled in spermatogonia has been unknown. We have found that the Doublesex-related transcription factor DMRT1 determines whether spermatogonia undergo mitosis or initiate meiosis. Spermatogonia lacking DMRT1 have abnormally active RA signaling and prematurely enter meiosis, independent of the normal spermatogenic cycle. Chromatin immunoprecipitation and other approaches show that control of meiotic initiation by DMRT1 involves direct transcriptional regulation of key RA metabolic enzymes and *Stra8*. Analysis of vitamin A depleted animals that lack RA reveals that DMRT1 also controls at least one retinoid-independent meiotic inducer. These results establish DMRT1 as an essential and direct regulator of the mitosis versus meiosis switch. The DM domain gene family to which DMRT1 belongs is deeply conserved in metazoan sexual regulation, and thus our findings also may have implications for meiotic control outside of mammals.

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Program/Abstract # 343

The RNA-binding protein Nanos2 is required to maintain spermatogonial stem cells

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In mice, spermatogenesis is initiated from a small number of stem cells belonging to undifferentiated spermatogonia. However, it remains unclear 1) which types of spermatogonia actually act as the stem cells and 2) how is the stem cell function regulated. Nanos, a zinc-finger RNA-binding protein, has been proposed as a conserved factor for germline stem cell function. In adult testes, Nanos2 is predominantly expressed in a subset of undifferentiated spermatogonia. However, the majority of *Nanos2*-null germ cells die by apoptosis before birth, hindering functional studies of Nanos2 during spermatogenesis. With the use of transgenic mouse strategies, I found that the RNA-binding protein Nanos2 is a key regulator for the maintenance of spermatogonial stem cells. Lineage-tracing analyses revealed that *Nanos2*-expressing spermatogonia self-renew and generate the entire spermatogenic cell lineage. Conditional disruption of postnatal *Nanos2* depleted spermatogonial stem cell reserves, whereas mouse testes in which *Nanos2* had been overexpressed accumulated spermatogonia with undifferentiated, stem cell-like properties. Thus, Nanos2 is expressed in self-renewing spermatogonial stem cells and maintains the stem cell state during murine spermatogenesis.

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Program/Abstract # 344

Stage-specific expression of the homeodomain protein Cux1 in Sertoli cells and spermatids during spermatogenesis

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The homeodomain protein Cux1 exists as multiple isoforms. The 200 kDa Cux1 protein is highly expressed in the developing kidney, where it functions to regulate cell proliferation. A 55 kDa Cux1 isoform is expressed exclusively in the testes. Transgenic mice ectopically expressing the 200 kDa Cux1 protein develop transient multiorgan hyperplasia, including the testes. We determined the pattern and timing of Cux1 protein expression in the developing testes. Cux1 expression was continuous in Sertoli cells of prepubertal testes, but became cyclic when spermatids appeared. In mature mice, Cux1 was highly expressed only in round spermatids at stages IV–V of spermatogenesis, in both spermatids and Sertoli cells at stages VI–X, and only in Sertoli cells at Stage XI. In Cux1 transgenic mice there were significantly fewer tubules expressing Cux1 in both Sertoli cells and spermatids and significantly more tubules expressing Cux1 in either spermatids or Sertoli cells. Moreover, Cux1 was not expressed in proliferating cells in testes from either wild type or transgenic mice. Thus, unlike the role of the somatic form of Cux1 in cell proliferation, the testis-specific form of Cux1 is not involved in cell division and appears to play a role in signaling between spermatids and Sertoli cells.

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Program/Abstract # 345

Inhibitory action of *Xenopus* dicalcin on sperm–egg interaction during fertilization

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To contribute to the study of sperm–egg interaction in the course of fertilization, we have isolated and characterized *Xenopus* dicalcin in *Xenopus* eggs. *Xenopus* dicalcin is localized markedly in the egg-coating envelope (called vitelline envelope; VE), and exhibits a Ca^{2+}-dependent binding to two glycoproteins that constitute polymeric filaments of VE. Since these VE glycoproteins are considered to function as sperm-receptors, we examined the effect of dicalcin on sperm–VE binding, sperm–VE penetration, and fertilization *in vitro*. Preincubation of *Xenopus*